FEBS Letters 586 (2012) 4326–4331

journal homepage: www.FEBSLetters.org

Depletion of CSN5 inhibits Ras-mediated tumorigenesis by inducing premature senescence in p53-null cells

Ikuko Tsujimoto, Akihiro Yoshida, Noriko Yoneda-Kato, Jun-ya Kato *

Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan

ARTICLE INFO

Article history:
Received 6 September 2012
Revised 2 October 2012
Accepted 23 October 2012
Available online 2 November 2012

Edited by Angel Nebreda

Keywords:
CSN5
COP9 signalosome
Ras
p53
Tumorigenesis
Senescence
Cell cycle

ABSTRACT

The mammalian COP9 signalosome (CSN) complex is involved in cell transformation, but its molecular mechanism remains undetermined. Here we show that disruption of the fifth component (CSN5) prevented the formation of tumors by p53-null cells transformed with an active form of Ras in subcutaneously injected mice. Depletion of CSN5 suppressed cell proliferation, and induced premature senescence characterized by upregulation of senescence-associated-β-galactosidase activity and increased expression of CDK inhibitors. CSN5-depleted cells exhibited enhanced activation of the PI3 kinase–Akt pathway, and chemical inhibition of this pathway reduced the level of senescence. Thus, CSN5 is suggested to be a novel target in cancer therapy and for drugs against tumor cells harboring mutated p53.

1. Introduction

The small GTP-binding protein Ras plays a central role in signal transduction mediated by growth factors and in cell transformation [1]. Although ectopic expression of constitutively active Ras in primary cells induces premature senescence and eternal withdrawal from the cell cycle [2], simultaneous inactivation of the tumor suppressor p53 combined with Ras produces transformed cells, which form tumors when injected into experimental animals [3]. Basically, Ras mediates signaling triggered by growth factor receptors, including a Raf/MEK/ERK pathway (MAPK pathway), and a PI3 kinase/Akt kinase pathway (PI3K–Akt pathway), both of which play an essential role in controlling the proliferation, differentiation, and survival of mammalian cells [1]. Many factors have been demonstrated to act downstream of and in parallel with Ras-mediated oncogenic pathways, among which we focused on the mammalian COP9 signalosome (CSN) complex [4,5]. The CSN complex consists of 8 subunits (CSN1–8), and knockdown of the subunits by siRNA prevented Ras-mediated transformation [6]. However, the precise mechanism remained to be determined.

The CSN complex exhibits multiple functions, which includes the deneddylase activity [7–9]. The deneddylase is an isopeptidase, which removes a ubiquitin-like peptide called NEDD8 from the cullin subunit of cullin-RING ubiquitin ligases (CRLs), the largest family among the ubiquitin ligases, thereby regulating ubiquitination-dependent proteolysis through the 26S proteasome. The fifth subunit of the CSN complex (CSN5, also known as Jab1) plays an important role in deneddylation; the JAMM domain located in the N-terminus of CSN5 is essential to this function [4,5], presumably serving as a catalytic core of the enzyme. However, the deneddylation reaction requires the holo–CSN complex. Recently, bortezomib (Velcade) and thalidomide, chemical inhibitors of the proteasome and a CRL complex containing cereblon, respectively, were developed as an anti-cancer drug for multiple myelome [10–12], indicating that this proteolytic pathway is a novel target for anti-cancer drugs, and also suggest that the upstream regulator of this pathway is a potential target for cancer treatment. In fact, an inhibitor of NEDD8-activating enzyme is a promising anti-cancer reagent [13]. The CSN complex may also be a good target for anti-cancer treatment.

We have previously found that in cells originating from Chronic Myeloid Leukemia (CML), the smaller form of CSN5 was accumulated depending on the activity of Bcr-Abl kinase and knockdown of CSN5 prevented proliferation of Bcr-Abl-transformed cells [14]. In this signaling, we also found that both PI3 kinase and MAP kinase pathways functioned upstream of the CSN complex. CSN5 is overexpressed in a variety of human cancers, and its specific knockdown prevented proliferation of tumor cells [5]. In animal

* Corresponding author. Address: Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan. Fax: +81 743 72 5519.
E-mail address: jkata@bs.naist.jp (J.-y. Kato).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2012.10.042

models, CSN5-transgenic mice developed myeloproliferative disorders [15], and knockout of the CSN5 allele resulted in embryonic lethality at a very early stage [16]. Conditional knockout showed that CSN5-depletion induced premature senescence in the presence and absence of p53 [17]. Based on these findings, we sought the physiological role of CSN5 in Ras-mediated tumorigenesis through the inducible knockout of the CSN5 gene in cells transformed using a combination of the nullizygous allele of the p53 tumor suppressor and the ectopic introduction of a constitutively active form of the Ras oncogene. We found that CSN5-knockout prevented the formation of tumors by p53-null Ras-transformed cells in mice by inducing premature senescence. CSN5-depleted cells were positive for senescence-associated-β-galactosidase activity, and had increased levels of ARF, and CDK inhibitors p16, p27, and p21. Interestingly, the PI3-kinase/Akt pathway, but not the MAP kinase pathway, was enhanced, and treatment with a PI3 kinase inhibitor, wortmannin, partially prevented senescence. Thus, CSN5 is an important component for Ras-mediated cell transformation by preventing premature senescence.

2. Materials and methods

2.1. Cell culture, transfection, and retroviral infection

Mouse embryonic fibroblasts (MEFs) harboring null (−) and conditional (floxed, f) alleles of the CSN5/Jab1 gene and immortalized by the null allele of p53 were described previously [16,17]. NIH3T3 (Arf-null, p53-wild-type) mouse fibroblasts (provided by Drs. C.J. Sherr and M.F. Roussel), MEFs, and 293T human embryonic kidney (HEK) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 100 units/ml of penicillin, and 100 μg/ml of streptomycin (GIBCO/BRL). Transfection with expression vectors was performed by the calcium phosphate–DNA precipitation method [18]. For viral production, the plasmid was co-transfected into 293T cells together with a plasmid encoding an ecotropic helper virus containing a defective virion-packaging (φ2) sequence. Culture supernatants containing retroviruses were used to infect mouse fibroblasts [19]. Infected cells were selected for resistance to puromycin (5 μg/ml) or G418 (1 mg/ml) depending on the type of vector used for infection and sorted for GFP-positive signals with a FACS Aria flow cytometer (Becton Dickinson). For the activation of inducible CRE proteins, MEFs were treated with 4OHT (200 nM) for 1–4 h. High molecular DNA was extracted from the cells and genotypes were determined as described [17]. DNA content and BrdU uptake were measured as described [19,20]. β-Galactosidase activity was determined as described [17]. In some experiments, cells were incubated in the presence of wortmannin (100 nM) and LY294002 (20 μM) before harvest.

2.2. Plasmid construction

The GFP-fused protein expression vectors (pMSCV-puro-GFP and pMSCV-IRES-GFP, the latter being a gift from Dr. Owen Witte), into which CRE-ER, ER-CRE-ER (purchased from Addgene Inc.) [21] and active Ha-Ras (EJ-ras) [22] cDNAs were subcloned, were described previously [19]. The vectors for RNA interference (RNAi) were constructed with the pSUPER RNAi system (pSUPER.retro, pSUPER.retro.neo+gfp, and pSUPER.retro.neo, purchased from OligoEngine) according to the manufacturer's instructions.

2.3. Protein analyses

Cell lysis, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and immunoblotting were performed as described [19,23]. Rabbit polyclonal antibodies to CSN5, Cul1 and 4, p21, GFP, and Skp2 were generated using bacterially produced polypeptides in our laboratory. Rabbit polyclonal antibodies to Skp2 (H-435), p27 (C-19), p16 (M156), Erk1 (C-16), Erk2 (C-14), and ppRb (S780) were obtained from Santa Cruz. Mouse monoclonal antibodies to γ-tubulin (GTU88) and Rb (G3-245) were purchased from Sigma and Pharmingen, respectively. Rabbit polyclonal antibodies to Akt (C67E7), phosphorylated Akt (C31E5E and D9E), and phosphorylated Akt-substrates (100B7E and #9611) were acquired from Cell Signaling Technology. Mouse monoclonal antibody to phosphorylated Erk1/2 (pT202/pY204, 20A) and rabbit polyclonal antibody to p19ARF (ab80) were from BD Transduction Lab and Abcam, respectively.

2.4. Tumorigenicity assay

Cells (ca 10^6) were subcutaneously injected into 6-week-old NOD-SCID mice. Tumors were formed after 8 days. Mice were sacrificed and the size of the tumor was measured after 10 days post-injection.

3. Results

3.1. Inducible, conditional knockout of CSN5 in Ras-transformed p53-null cells

To examine the role of CSN5 in Ras-transformed cells, we conditionally knocked out the CSN5 gene. Because it is difficult to maintain CSN5-knockout cells for a long period, we utilized an inducible system in cultured cells to synchronously deplete the CSN5 protein. Mouse embryonic fibroblasts (MEFs) containing the floxed allele of the CSN5 gene and immortalized by the null allele of p53 ($CSN5^{f/-}p53^{-/-}$MEFs) [17] were infected with a retroviral vector encoding a constitutively active Ras and a vector containing inducible CRE recombinase, which was fused with the estrogen receptor (ER) gene (CRE-ER) [21]. After selection in puromycin and by cell sorting for GFP-positive signals, 4-Hydroxytamoxifen (4OHT) was added to the medium to inactivate the CSN5 locus. Fig. 1A shows that addition of 4OHT efficiently converted the floxed allele to the deleted form. Incubation of cells with 4OHT for 1–3 h was sufficient to convert all the detectable amount of floxed allele into the deleted allele, but it took a little longer for the CSN5 protein to disappear, and after 3 days, the protein was undetectable (Fig. 1B). After 7 days, a trace amount of the protein reappeared. This was due to the proliferation of cells that failed to convert the floxed allele of the CSN5 gene.

3.2. Depletion of CSN5 prevented tumor formation in mice

To confirm that the CSN activity is required for Ras-transformed cells to form tumors in vivo, we subcutaneously injected the cells prepared as in Fig. 1A and B ($CSN5^{f/-}p53^{-/-}Ras^{+}$CRE-ER MEFs) with and without incubation in 4OHT into NOD-SCID mice (Fig. 1C and D). Tumors were formed after 8 days, and mice were sacrificed and the size of the tumors was measured after 10 days post-injection. In contrast to the control (−4OHT), deprivation of the CSN5 protein induced by treatment with 4OHT (+4OHT) markedly reduced the size of the tumors (Fig. 1C and D). A trace amount of cells remained in mice at 10 days post-injection but usually failed to proliferate thereafter. In rare cases, we observed cell proliferation after 14 days post-injection, but genotyping revealed that such cells retained non-converted floxed alleles, presumably due to a non-functioning CRE recombinase. Thus, these results confirmed that CSN5 is required for Ras-transformed cells to form tumors in mice.

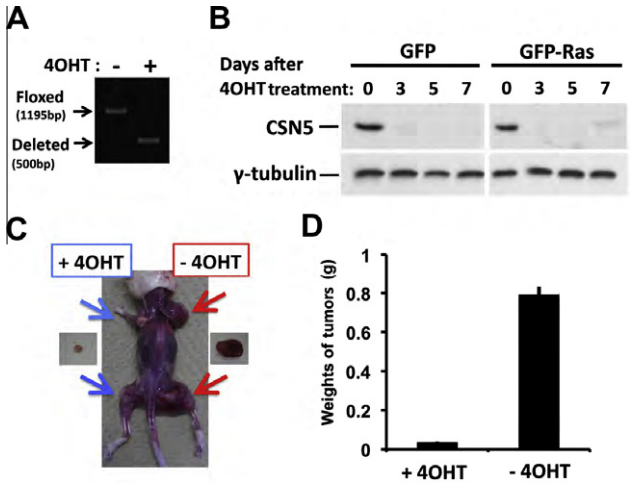

Fig. 1. Inducible knockout of the CSN5 gene prevented tumor formation in mice. (A) Mouse embryonic fibroblasts (MEFs) harboring the conditional floxed locus of CSN5 and the null locus of p53 and introduced with a constitutively active Ras and an inducible CRE (CRE-ER) (CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs) were incubated with and without 4OHT for 3 h. High molecular weight DNA was extracted from cells, and the genotypes were determined by genomic PCR as described [17]. (B) MEFs were prepared as in A, except that a control (GFP) virus was also used in lieu of active Ras as a control. Cells were incubated in 4OHT, and harvested 0, 3, 5, and 7 days after treatment. Cell lysates were analyzed by immunoblotting with antibodies against CSN5 and γ-tubulin. (C) CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs were cultured with and without 4OHT and subcutaneously injected into NOD-SCID mice (ca 10^6 cells). At 10 days post-injection, mice were sacrificed and the weights of the tumors were measured. The sites of injection are shown by arrows, and photos of actual tumors removed from mice are also shown. (D) The summary of 4 independent experiments of panel C is shown. The data are averages and standard deviations. $P < 0.001$.

3.3. Depletion of CSN5 prevented the proliferation and induced the premature senescence of p53$^{-/-}$ cells containing active Ras

To investigate how depletion of CSN5 prevented tumor formation, we analyzed their proliferation potential in vitro. CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER and control (infected with a control GFP virus in lieu of Ras virus) MEFs were incubated with and without 4OHT, plated at low cell density, and enumerated every day for 6–10 days. Fig. 2A shows that depletion of CSN5 markedly suppressed cell proliferation in the presence and absence of active Ras. Because Ras-transformed cells proliferated to some extent for a week whereas control cells ceased to proliferate in 2–3 days, we replated the 4OHT-treated Ras-transformed cells at Day 4, and enumerated them for a week (Fig. 2B), confirming that the cells ceased to proliferate in the end. In addition, we cultured Ras-transformed and control cells at Day 5 and 7 after treatment with 4OHT in the presence of BrdU and enumerated BrdU-positive cells (Fig. 2C). We found that CSN5-depletion inhibited the incorporation of BrdU in both Ras-transformed and control cells but Ras-transformed cells were slightly more resistant, consistent with the growth curve (Fig. 2A and B). During this period of culture, we did not observe any significant increase in the death and detachment of cells, suggesting that suppression of cell proliferation is due not to an increase in cell death or loss from floatation, but the blocking of cell proliferation. Flow-cytometric analysis of DNA content showed little difference in distribution among the G0/G1, S, and G2/M phases before and after 4OHT treatment (Fig. 2D), suggesting that the cell cycle was blocked at multiple points [17].

Under the microscope, 4OHT-treated cells appeared flatter (Fig. 2E, upper panels), a typical feature of senescent cells. We, therefore, assayed for senescence-associated (SA) β-galactosidase (Gal) activity, another marker of premature senescence. Fig. 2E, lower panels show that 4OHT-treated CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs were positive for SA-β-Gal activity. Thus, we concluded that depletion of CSN5 induced premature senescence in Ras-transformed cells to prevent tumorigenesis.

3.4. CSN5-depletion upregulated the expression of several senescence-associated marker molecules and sustained Akt signaling

To examine the changes in signaling molecules caused by CSN5-depletion, we analyzed the expression of a series of signaling molecules. CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs were cultured with and without 4OHT, incubated without 4OHT for 7 days, and harvested. The expression of each signaling molecule was determined by immunoblotting (Fig. 3). 4OHT-treatment did not significantly change the level of ectopic Ras expression, and, consistent with the loss of CSN5 expression, neddylated cullins were accumulated (Fig. 3A, first 4 lanes from the top). As cells underwent senescence, the expression of the CDK inhibitors p21, p27, and p16 was upregulated and hypo-phosphorylated Rb protein was accumulated, whereas the level of Skp2 was maintained (Fig. 3A, lanes 5–10 from the top). Interestingly, the level of p21 was slightly raised in these p53-null cells, probably due to the massive inactivation of cullin-containing ubiquitin ligases.

Because we previously showed that MAP kinase and PI3 kinase pathways are involved in regulation of the CSN complex depending on the activity of Bcr-Abl kinase [14], we examined the activation of these pathways in CSN5-depleted Ras-transformed cells by looking at ERK and Akt kinases. Fig. 3B shows that, although the total expression levels of ERK1, ERK2, and Akt were maintained, their activating phosphorylation was modulated by knockout of the CSN5 gene. The active form of ERK1 and 2 was reduced, whereas the activating phosphorylation of Akt (serine 473 and threonine 308) was increased after treatment with 4OHT. Furthermore, the phosphorylation of certain substrates of Akt was enhanced in cells deprived of CSN5 (Fig. 3C), demonstrating that substrates of Akt as well as Akt kinase itself were phosphorylated to a great extent in these cells.

3.5. Inhibition of the PI3 kinase–Akt pathway partially prevented premature senescence in CSN5-depleted cells

As activation of the PI3 kinase–Akt pathway was reported to induce senescence [24], we investigated the potential role of the increased activity of this pathway in CSN5-depleted Ras-transformed cells using a chemical inhibitor specific to PI3 kinase, wortmannin. CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs were treated with 4OHT, incubated without 4OHT for 7 days, and harvested. Wortmannin was added to the medium for the last 48 h before harvest. Analysis of the cell lysate by immunoblotting (Fig. 4A) showed that the activating phosphorylation of Akt at serine 473 and threonine 308 was enhanced by deprivation of CSN5 but suppressed in the presence of wortmannin (Fig. 4A, fouth and fifth lanes from the top). Under these conditions, levels of p27, p16, ARF and p21 were increased by treatment with 4OHT but not significantly affected by wortmannin (Fig. 4A, sixth to ninth lanes from the top). The amount of Skp2 was affected by neither 4OHT nor wortmannin (Fig. 4A, tenth lane from the top). In terms of morphology and SA-β-Gal activity (Fig. 4B), cells became flat and positive for SA-β-Gal activity following the treatment with 4OHT, but these phenotypes were markedly weakened in the presence of wortmannin. However, the presence of wortmannin did not allow cells to proliferate after treatment with 4OHT. We observed the same phenotype when we used a chemical inhibitor more specific to PI3 kinase, LY294002. Thus, these results show that upregulation of Akt (or PI3 kinase) activity played an important role in the induction of premature senescence induced by depletion of CSN5.

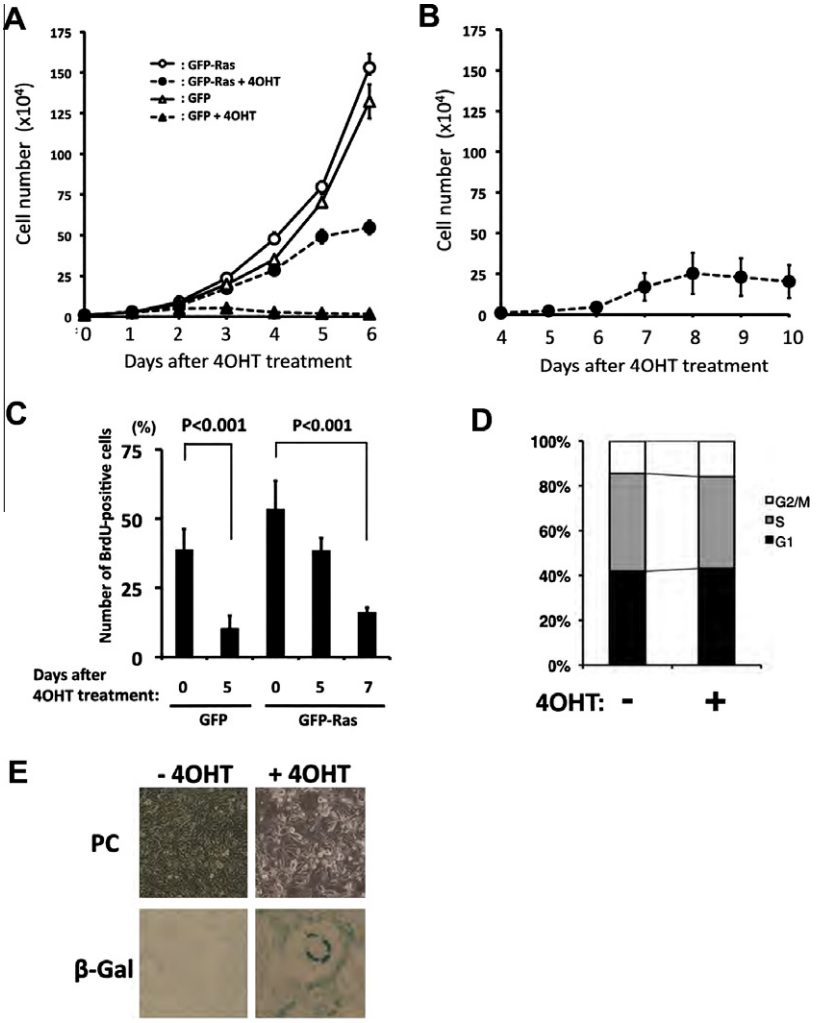

Fig. 2. Depletion of CSN5 prevented proliferation and induced premature senescence of cells containing active Ras and nullizygous p53. (A) CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs and control MEFs were incubated with and without 4OHT, replated at low cell density (2.5×10^4 cells per plate) and enumerated every day for 6 days. (B) CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs treated with 4OHT in panel A were replated at Day 4 and enumerated for another 6 days. (C) CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER (GFP-Ras) and control (CSN5$^{f/-}$p53$^{-/-}$GFP^{+}CRE-ER , GFP) MEFs were incubated with and without 4OHT, and replated at low cell density on cover slips. At Day 0, 5, and 7, cells were pulse-labeled with BrdU and stained with antibody to BrdU. Cells with positive signals for BrdU incorporation were enumerated. (D) CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs were incubated with and without 4OHT. After 7 days, DNA content was analyzed by flow cytometry. (E) CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs cultured with and without 4OHT and then incubated in normal medium for 7 days were observed under the microscope (phase contrast, PC), and stained for senescence-associated β-galactosidase activity (β-Gal). The data in A–C are averages and standard deviations for three independent experiments.

4. Discussion

Senescence has been recently recognized as part of the mechanism to prevent tumor development by suppressing and eventually removing transformed cells from the system [25]. In general, induction of senescence depends on the activity of the tumor suppressor p53 and its downstream targets such as the CDK inhibitor p21, and, actually, p53-null cells escape from replicative senescence and proliferate indefinitely [25,26]. However, recent research identified novel pathways, by which cells undergo senescence in a p53-independent manner. ARF induced p53-independent senescence in melanoma cells [27]. p16INK4a plays a critical role in p53-independent telomere-directed senescence [28]. Skp2 knockout induced Arf/p53 independent cellular senescence in PTEN-deficient tumor cells [29]. In this process, upregulation of CDK inhibitors p27 and p21 caused by Skp2-loss is thought to play an important role. Cdk4-loss induces Arf/p53-independent senescence to prevent oncogenic transformation [30]. Although the precise molecular mechanism acting to control p53-independent senescence remains to be determined, the finding that inactivation of Rb causes senescent cells to enter the cell cycle [31] suggests that the Rb protein plays an essential role in the initiation and maintenance of senescence as well as in progression through the G0/G1 phase, and p53 acts upstream of Rb through p21.

In the present study, we showed that depletion of CSN5 induced senescence in p53-null cells transformed by active Ras. We found that a hypo-phosphorylated Rb protein was accumulated in CSN5-depeleted cells (Fig. 5), consistent with the notion that dephosphorylation of Rb is involved in senescence. We also found that the activity of Akt was enhanced in CSN5-depleted cells and chemical inhibition of PI3 kinase by wortmannin reduced activating phosphorylation of Akt and suppressed premature senescence, suggesting that deregulated Akt acts on a novel target ultimately leading to induction of hypo-phosphorylation of Rb to initiate premature senescence.

Although Akt does induce senescence [24], it occurs through TORC1 and p53, and is presumably different from the p53-independent senescence induced by CSN5-depletion. CSN5-depletion

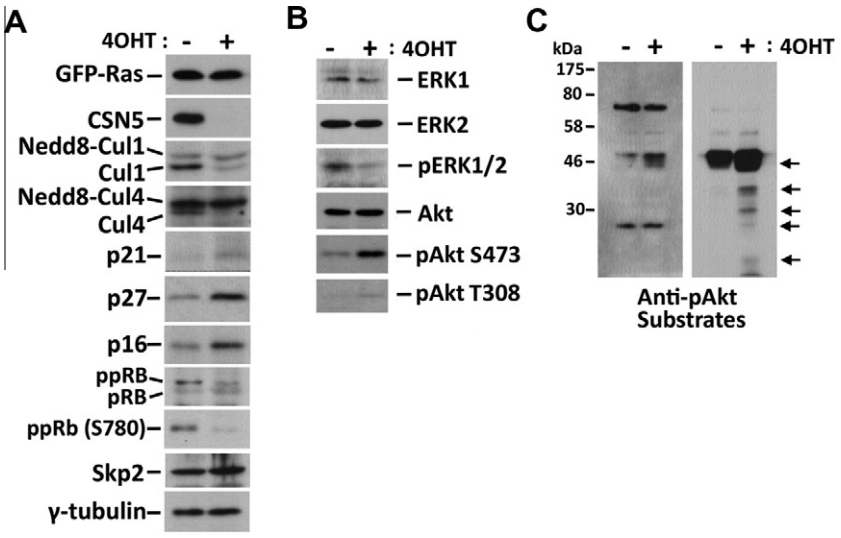

Fig. 3. Upregulation of senescence-associated CDK inhibitors and enhancement of Akt signaling in CSN5-depleted cells. Lysates from CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs cultured with and without 4OHT and then incubated in normal medium for 7 days were analyzed by immunoblotting with antibodies against GFP, CSN5, Cul1, Cul4, p21, p27, p16, pRb, phosphorylated Rb (S780), Skp2, γ-tubulin (A), ERK1, ERK2, phosphorylated ERK1/2, Akt, phosphorylated AKT (S473 and T308) (B), and phosphorylated Akt substrates (100B7E and #9611 for left and right panels, respectively) (C).

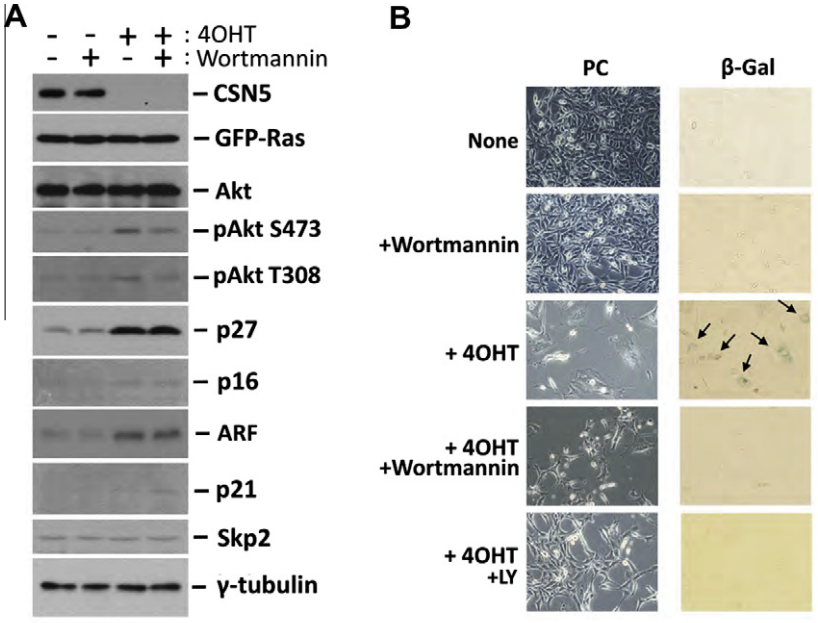

Fig. 4. Inhibition of the PI3 kinase–Akt pathway by wortmannin partially rescued CSN5-depleted cells from premature senescence. (A) CSN5$^{f/-}$p53$^{-/-}$Ras^{+}CRE-ER MEFs cultured with and without 4OHT were incubated in medium in the presence and absence of wortmannin. Cells were harvested and cell lysates were analyzed by immunoblotting with antibodies against CSN5, GFP, Akt, phosphorylated AKT (T473 and S308), p27, p16, ARF, p21, Skp2, and γ-tubulin. (B) MEFs prepared as in panel A were observed under the microscope (PC), and stained for senescence-associated β-galactosidase activity (β-Gal). Cells with positive signals for β-Gal are marked with arrows. The result of cells treated with LY294002 is also shown.

did induce upregulation of p16, p21, p27, and ARF expression, however, treatment with wortmannin reduced the level of senescence without altering the expression of p16, p21, p27, and ARF, suggesting that depletion of CSN5 triggers premature senescence via a novel mediator under the control of Akt.

In addition, it is known that arresting cells in G0/G1 is not sufficient to induce senescence and additional signal is required to push cells from G0/G1 arrest into the senescent state. The system used in this study may be ideal for investigating this unknown signaling pathway that executes the final step in the induction of senescence. Whatever the mechanism, it is feasible that a CSN5-associated function is required for the Ras-mediated transformation of cells to prevent premature senescence and, therefore, CSN5 is a novel target for development of drugs effective against cancer cells that harbor mutations in the p53 allele to prevent proliferation. However, total depletion of CSN5 results in a destruction of the holo–CSN complex, leading to pleiotropic defects on cell function including cell cycle arrest at multiple points, which will probably cause severe side effects. Therefore, it would be preferable to pinpoint the signaling pathway specifically associated with

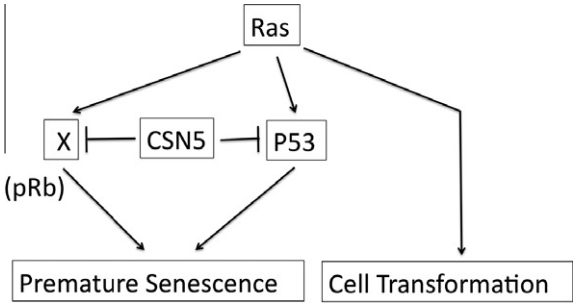

Fig. 5. A model of the control of senescence mediated by CSN5 in the Ras-mediated signaling pathway.

p53-independent premature senescence among multiple functions of CSN5, and manipulation of that specific factor or pathway will be a solution to the development of desirable drugs.

Acknowledgements

We thank Drs. C.J. Sherr and M.F. Roussel for the NIH3T3 cell line, Dr. O. Witte for the MSCV vector, Dr. R. Pardi for conditional knockout mice of CSN5, and Ms. I. Nakamae for excellent technical assistance. This work was supported by Grants-in-aid for Scientific Research and for Cancer Research from the Ministry of Education, Science, and Culture of Japan.

References

[1] Pylayeva-Gupta, Y., Grabocka, E. and Bar-Sagi, D. (2011) RAS oncogenes: weaving a tumorigenic web. Nat. Rev. Cancer 11, 761–774.
[2] Serrano, M., Lin, A.W., McCurrach, M.E., Beach, D. and Lowe, S.W. (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 88, 593–602.
[3] Parada, L.F., Land, H., Weinberg, R.A., Wolf, D. and Rotter, V. (1984) Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. Nature 312, 649–651.
[4] Wei, N. and Deng, X.W. (2003) The COP9 signalosome. Annu. Rev. Cell Dev. Biol. 19, 261–286.
[5] Kato, J.Y. and Yoneda-Kato, N. (2009) Mammalian COP9 signalosome. Genes Cells 14, 1209–1225.
[6] Luo, J., Emanuele, M.J., Li, D., Creighton, C.J., Schlabach, M.R., Westbrook, T.F., Wong, K.K. and Elledge, S.J. (2009) A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell 137, 835–848.
[7] Bech-Otschir, D., Seeger, M. and Dubiel, W. (2002) The COP9 signalosome: at the interface between signal transduction and ubiquitin-dependent proteolysis. J. Cell Sci. 115, 467–473.
[8] Cope, G.A. and Deshaies, R.J. (2003) COP9 signalosome: a multifunctional regulator of SCF and other cullin-based ubiquitin ligases. Cell 114, 663–671.
[9] Schwechheimer, C. and Deng, X.W. (2001) COP9 signalosome revisited: a novel mediator of protein degradation. Trends Cell Biol. 11, 420–426.
[10] Paramore, A. and Frantz, S. (2003) Bortezomib. Nat. Rev. Drug Discov. 2, 611–612.
[11] Singhal, S. et al. (1999) Antitumor activity of thalidomide in refractory multiple myeloma. N. Engl. J. Med. 341, 1565–1571.
[12] Ito, T., Ando, H., Suzuki, T., Ogura, T., Hotta, K., Imamura, Y., Yamaguchi, Y. and Handa, H. (2010) Identification of a primary target of thalidomide teratogenicity. Science 327, 1345–1350.
[13] Soucy, T.A. et al. (2009) An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature 458, 732–736.
[14] Tomoda, K., Kato, J.Y., Tatsumi, E., Takahashi, T., Matsuo, Y. and Yoneda-Kato, N. (2005) The Jab1/COP9 signalosome subcomplex is a downstream mediator of Bcr-Abl kinase activity and facilitates cell-cycle progression. Blood 105, 775–783.
[15] Mori, M., Yoneda-Kato, N., Yoshida, A. and Kato, J.Y. (2008) Stable form of JAB1 enhances proliferation and maintenance of hematopoietic progenitors. J. Biol. Chem. 283, 29011–29021.
[16] Tomoda, K., Yoneda-Kato, N., Fukumoto, A., Yamanaka, S. and Kato, J.Y. (2004) Multiple functions of Jab1 are required for early embryonic development and growth potential in mice. J. Biol. Chem. 279, 43013–43018.
[17] Yoshida, A., Yoneda-Kato, N., Panattoni, M., Pardi, R. and Kato, J.Y. (2010) CSN5/Jab1 controls multiple events in the mammalian cell cycle. FEBS Lett. 584, 4545–4552.
[18] Chen, C. and Okayama, H. (1987) High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. 7, 2745–2752.
[19] Yoneda-Kato, N., Tomoda, K., Umehara, M., Arata, Y. and Kato, J.Y. (2005) Myeloid leukemia factor 1 regulates p53 by suppressing COP1 via COP9 signalosome subunit 3. EMBO J. 24, 1739–1749.
[20] Tomoda, K. et al. (2002) The cytoplasmic shuttling and subsequent degradation of p27Kip1 mediated by Jab1/CSN5 and the COP9 signalosome complex. J. Biol. Chem. 277, 2302–2310.
[21] Matsuda, T. and Cepko, C.L. (2007) Controlled expression of transgenes introduced by in vivo electroporation. Proc. Natl. Acad. Sci. USA 104, 1027–1032.
[22] Fukumoto, A., Tomoda, K., Kubota, M., Kato, J.Y. and Yoneda-Kato, N. (2005) Small Jab1-containing subcomplex is regulated in an anchorage- and cell cycle-dependent manner, which is abrogated by ras transformation. FEBS Lett. 579, 1047–1054.
[23] Kato, J.Y., Nakamae, I., Tomoda, K., Fukumoto, A. and Yoneda-Kato, N. (2006) Preparation and characterization of monoclonal antibodies against mouse Jab1/CSN5 protein. Hybridoma (Larchmt) 25, 342–348.
[24] Astle, M.V. et al. (2012) AKT induces senescence in human cells via mTORC1 and p53 in the absence of DNA damage: implications for targeting mTOR during malignancy. Oncogene 31, 1949–1962.
[25] Acosta, J.C. and Gil, J. (2012) Senescence: a new weapon for cancer therapy. Trends Cell Biol. 22, 211–219.
[26] Collado, M., Blasco, M.A. and Serrano, M. (2007) Cellular senescence in cancer and aging. Cell 130, 223–233.
[27] Ha, L. et al. (2007) ARF functions as a melanoma tumor suppressor by inducing p53-independent senescence. Proc. Natl. Acad. Sci. USA 104, 10968–10973.
[28] Jacobs, J.J. and de Lange, T. (2004) Significant role for p16INK4a in p53-independent telomere-directed senescence. Curr. Biol. 14, 2302–2308.
[29] Lin, H.K. et al. (2010) Skp2 targeting suppresses tumorigenesis by Arf–p53-independent cellular senescence. Nature 464, 374–379.
[30] Zou, X., Ray, D., Aziyu, A., Christov, K., Boiko, A.D., Gudkov, A.V. and Kiyokawa, H. (2002) Cdk4 disruption renders primary mouse cells resistant to oncogenic transformation, leading to Arf/p53-independent senescence. Genes Dev. 16, 2923–2934.
[31] Sage, J., Miller, A.L., Perez-Mancera, P.A., Wysocki, J.M. and Jacks, T. (2003) Acute mutation of retinoblastoma gene function is sufficient for cell cycle re-entry. Nature 424, 223–228.